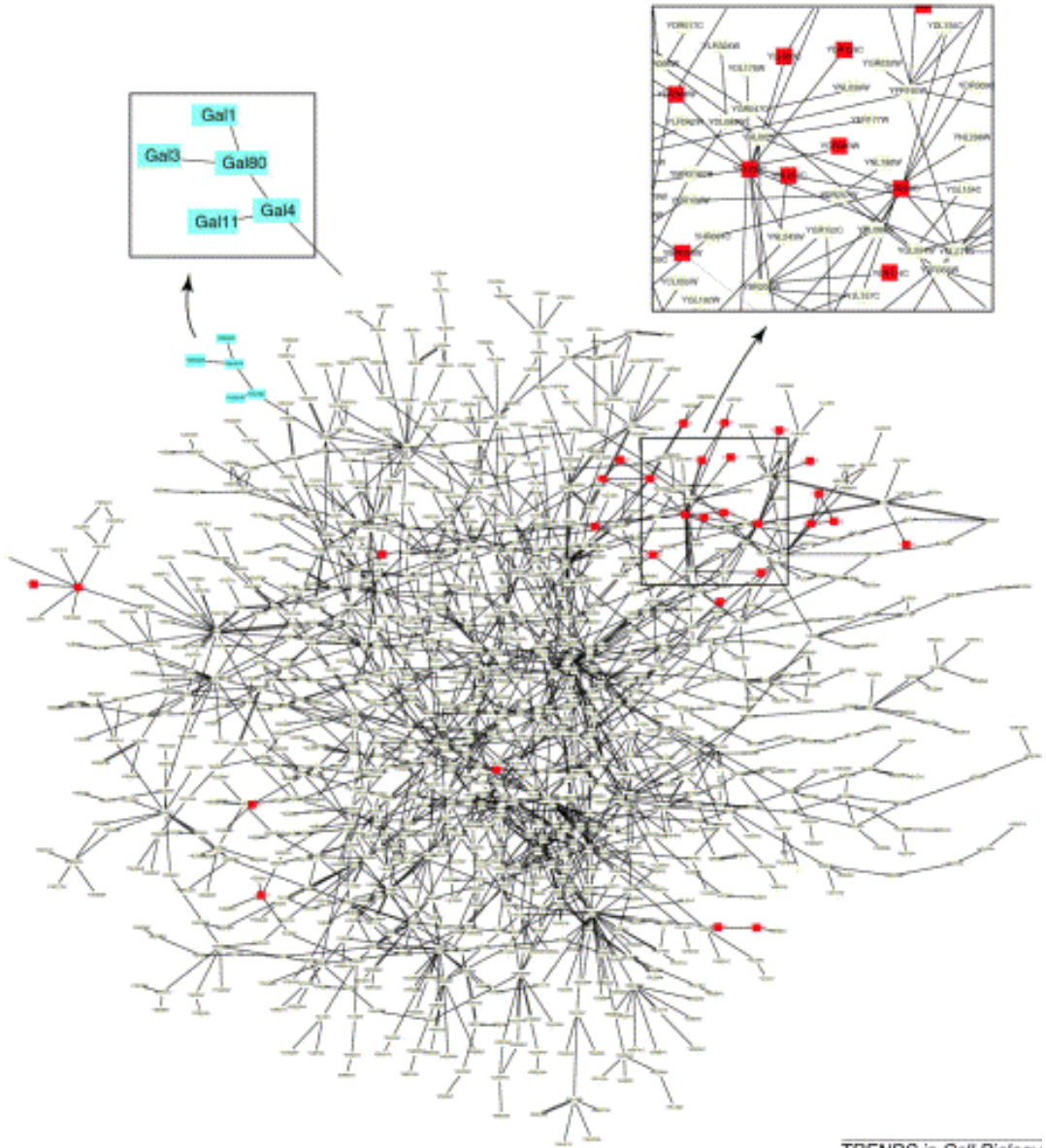
Simple Enzymatic Reaction:

$$S + E \leftrightarrow ES \rightarrow P + E$$

$$\text{Reaction Rate} = \frac{V_{\max}[S]}{K_m + [S]}$$

How do we relate to biology?



TRENDS in Cell Biology

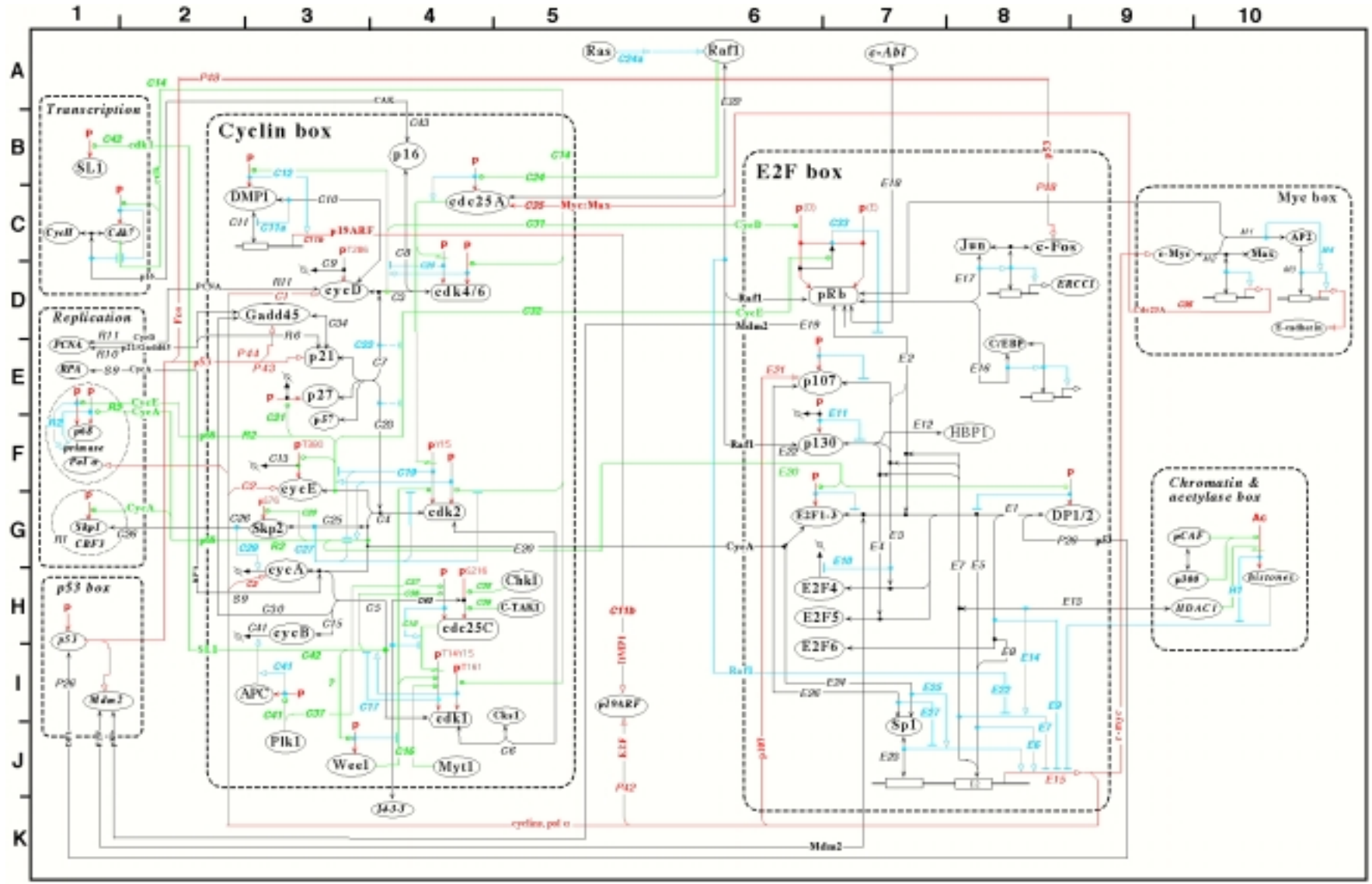
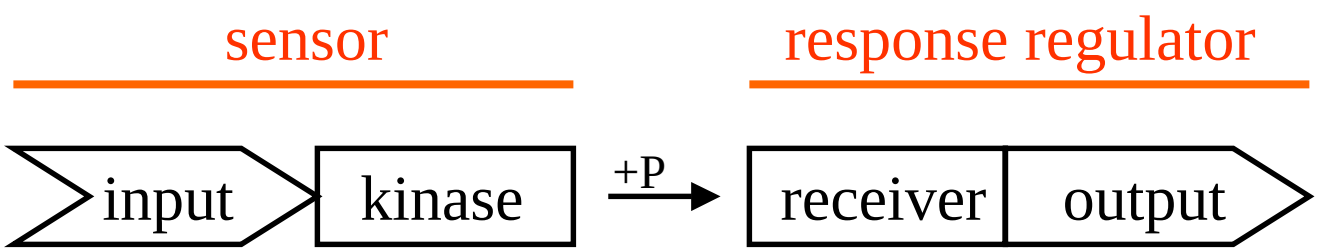


Figure 6A: The Cyclin - E2F cell cycle control system (version 3a - June 8, 1999)

Two Component Signaling



$$ATP + PK - His \leftrightarrow ADP + PK - His \sim P$$

$$PK \sim P + RR - Asp \rightarrow PK + RR - Asp \sim P$$

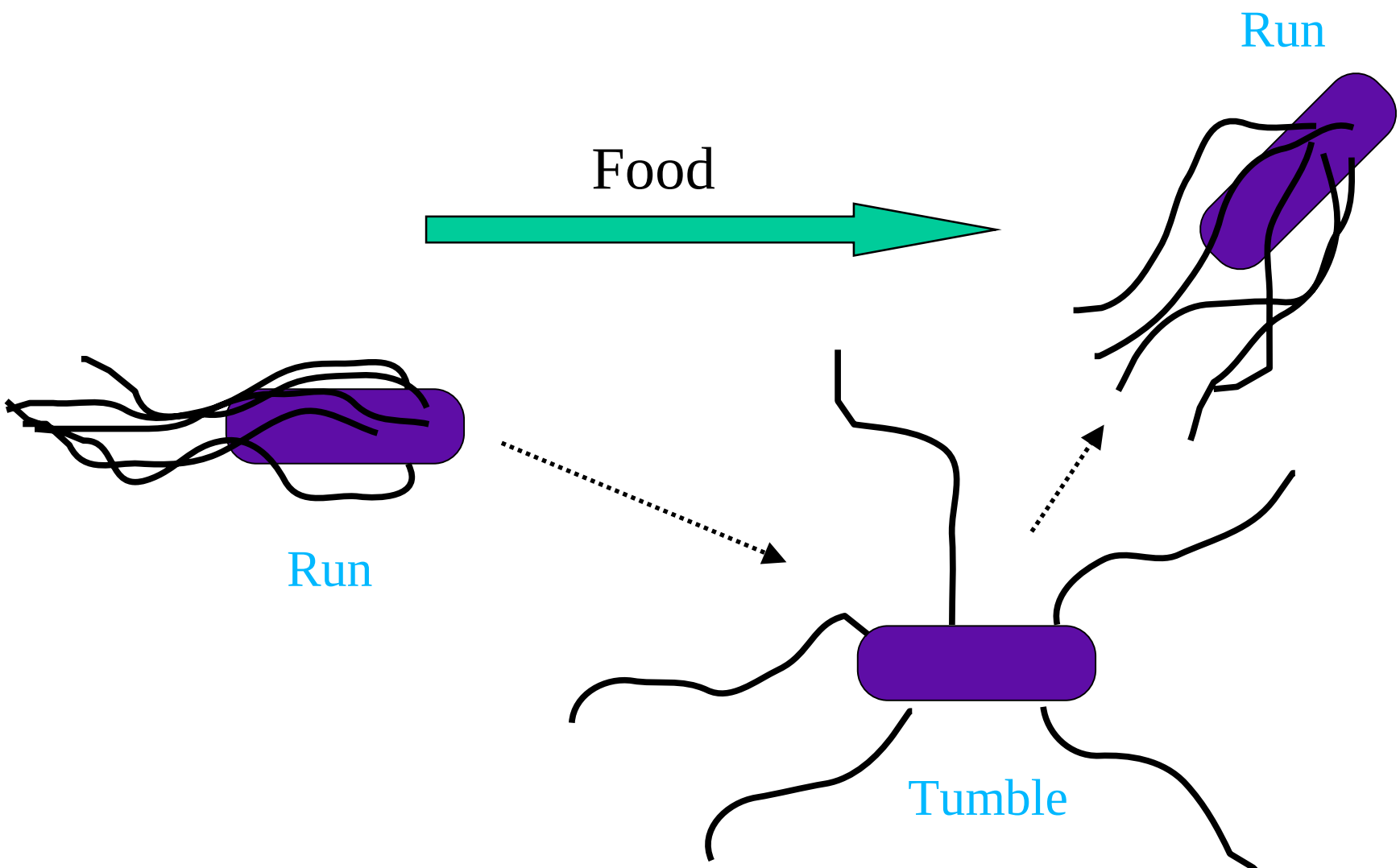
$$H_2 0 + RR - Asp \sim P \rightarrow RR - Asp + P$$

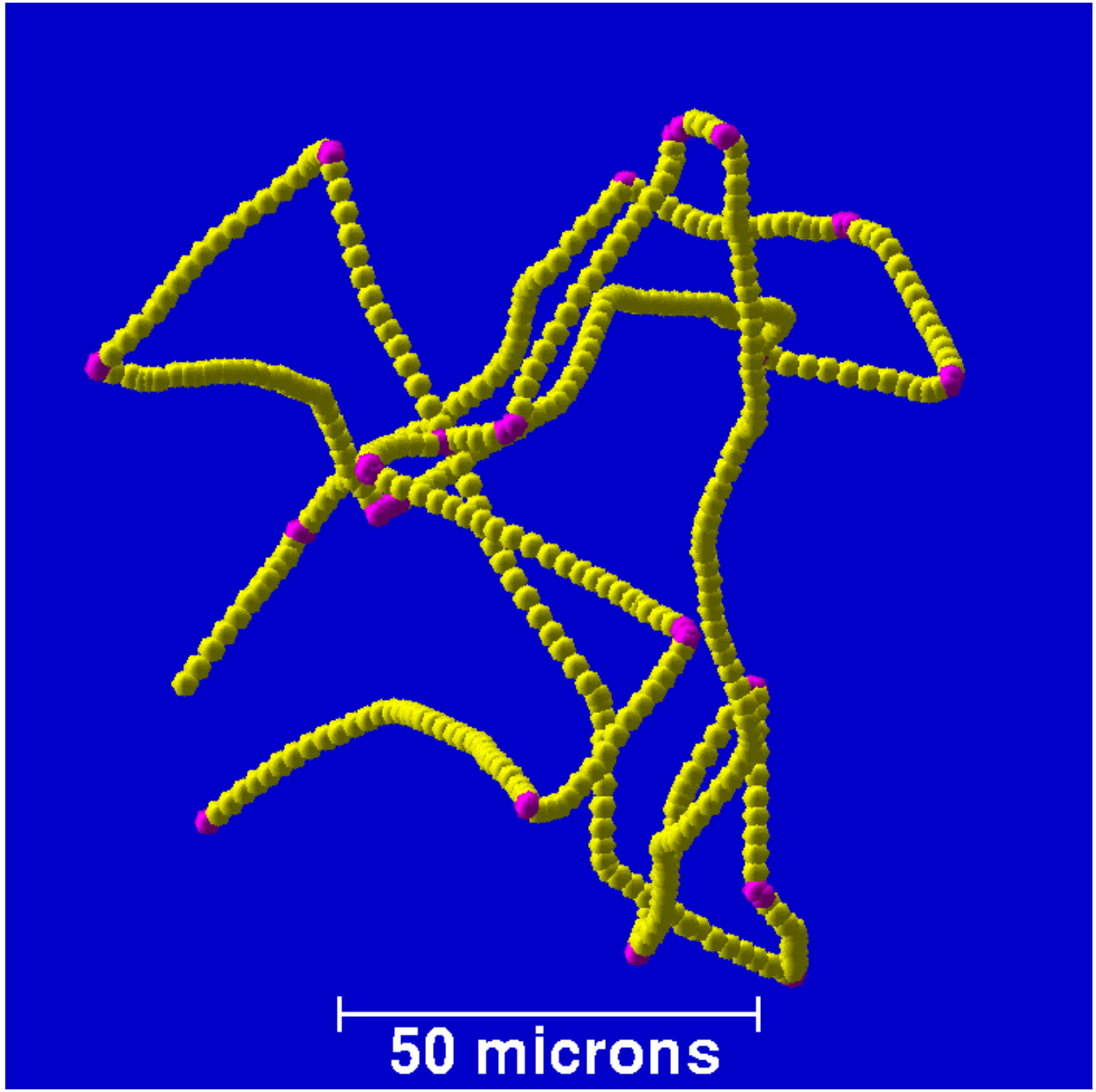
Examples of two-component systems in Bacteria

Chemotaxis
Nitrogen Regulation
Phosphate Regulation
Osmoregulation
Sporulation
Competence
Flagellar Biosynthesis
Oxygen Regulation
Salmonella Virulence
Tricarboxylate Transport
etc…

300+ Systems identified in 100+ organisms.
More than 30 known systems in *E. coli* and *B. subtilis*

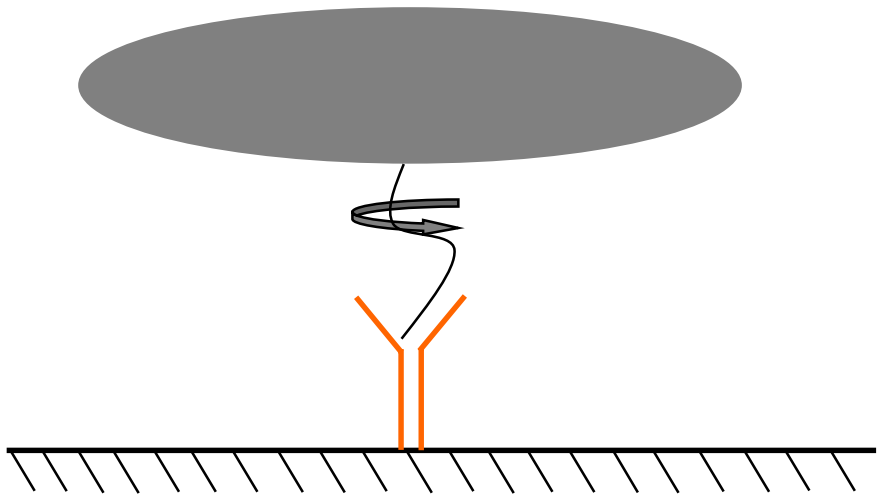
Chemotaxis



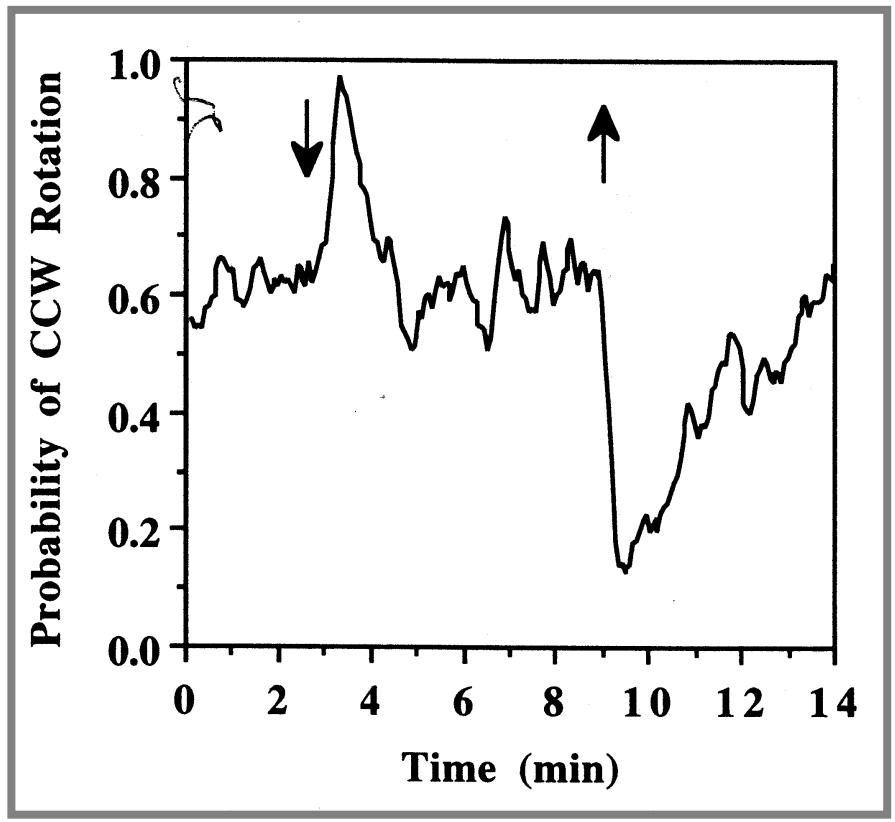


R.M. Ford (UVA)

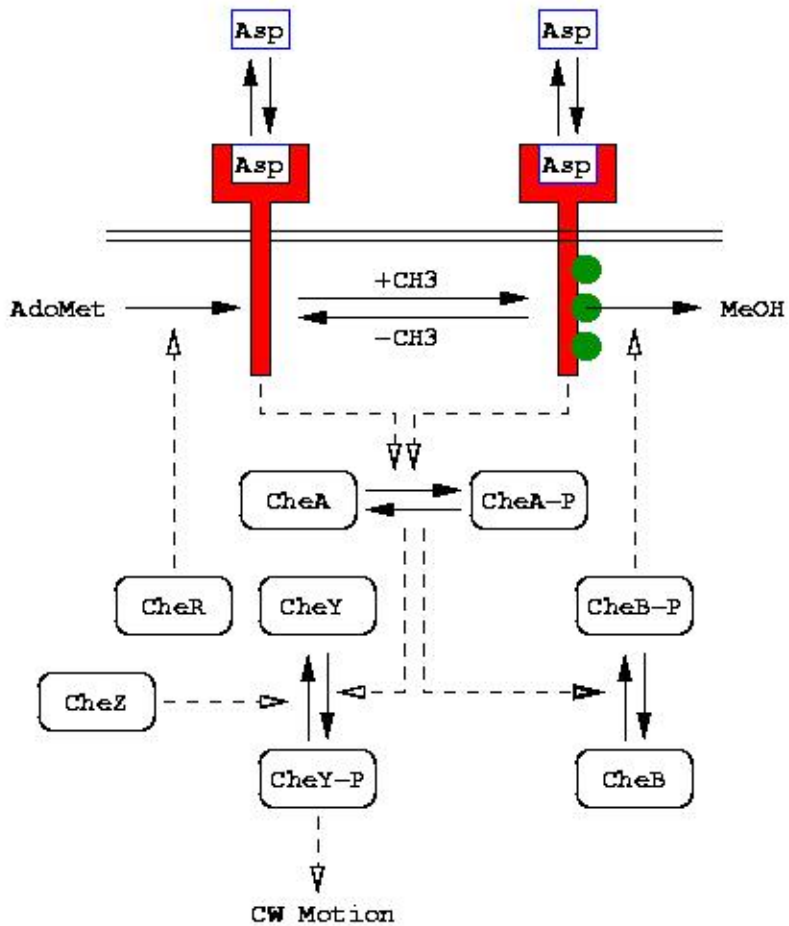
Tethering Analysis



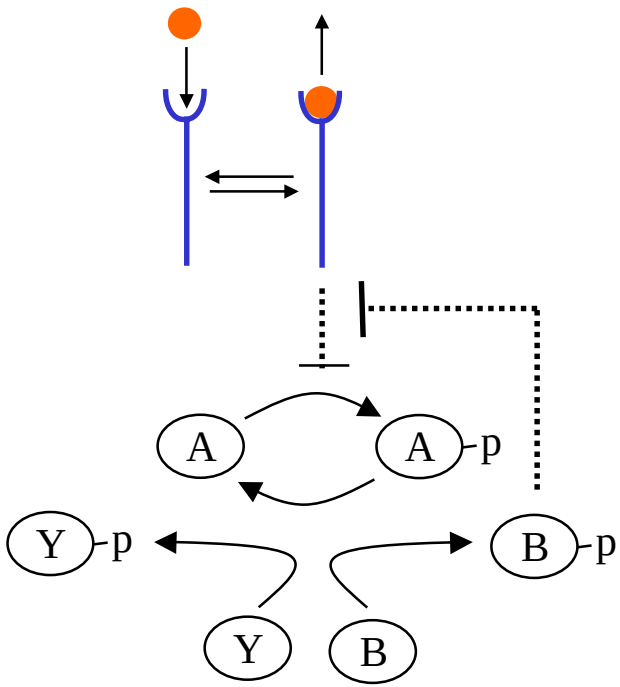
Adaptation


Probability of CCW Rotation
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
Time (min)

E. coli Chemotaxis Pathway



E. coli Control Diagram



Kinetic Model

$$Tar_N + L \leftrightarrow Tar_N L$$

$$A + Tar_N \rightarrow Ap + Tar_N$$

$$A + Tar_N L \rightarrow Ap + Tar_N L$$

$$Ap + Y \rightarrow A + Yp$$

$$Ap + B \rightarrow A + Bp$$

$$R + Tar_N \rightarrow Tar_{N+1}$$

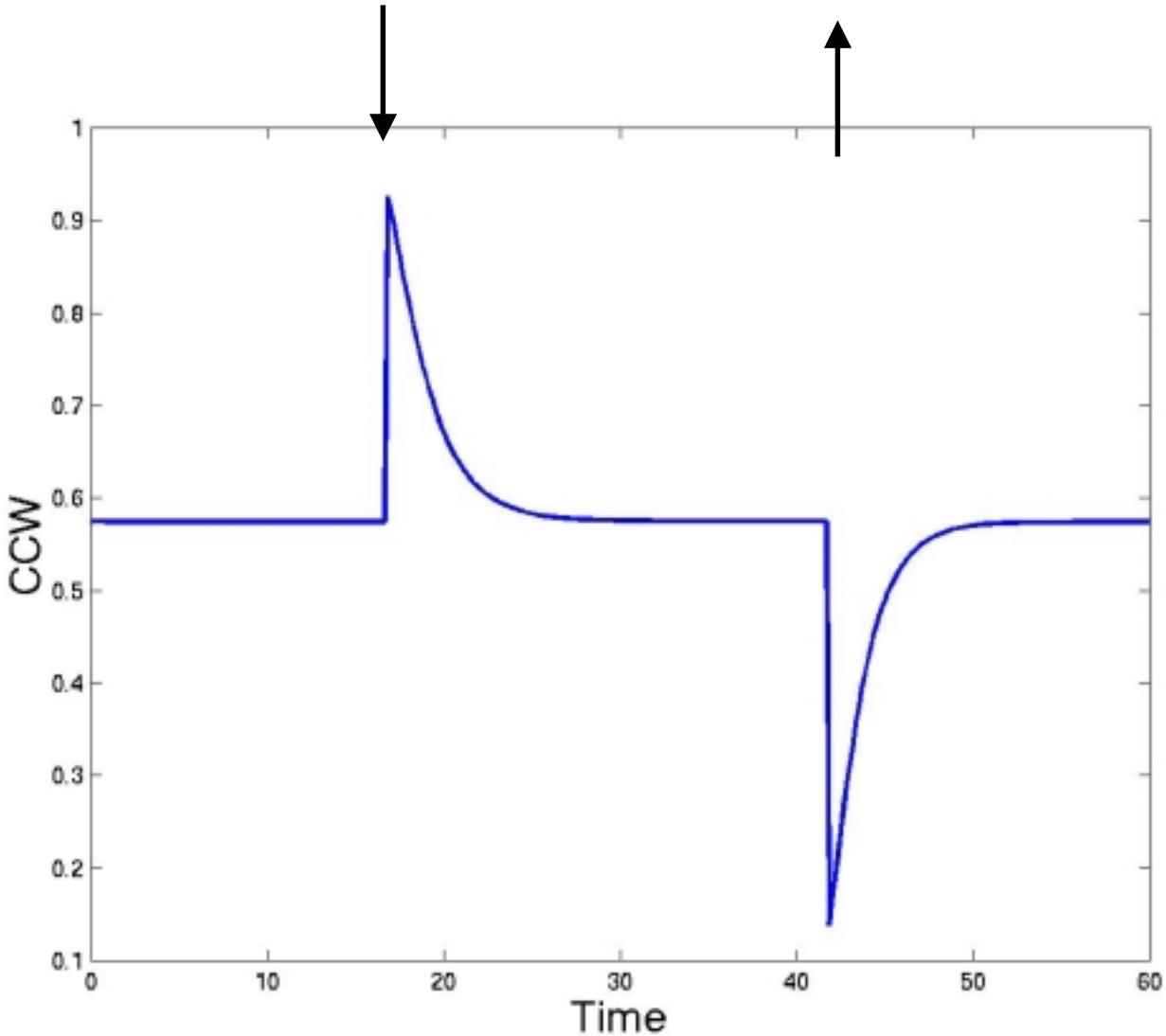
$$Bp + Tar_N \rightarrow Tar_{N-1} + Bp$$

$$R + Tar_N L \rightarrow Tar_{N+1} L$$

$$Bp + Tar_N L \rightarrow Tar_{N-1} L + Bp$$

$$Yp + Z \rightarrow Z + Y$$

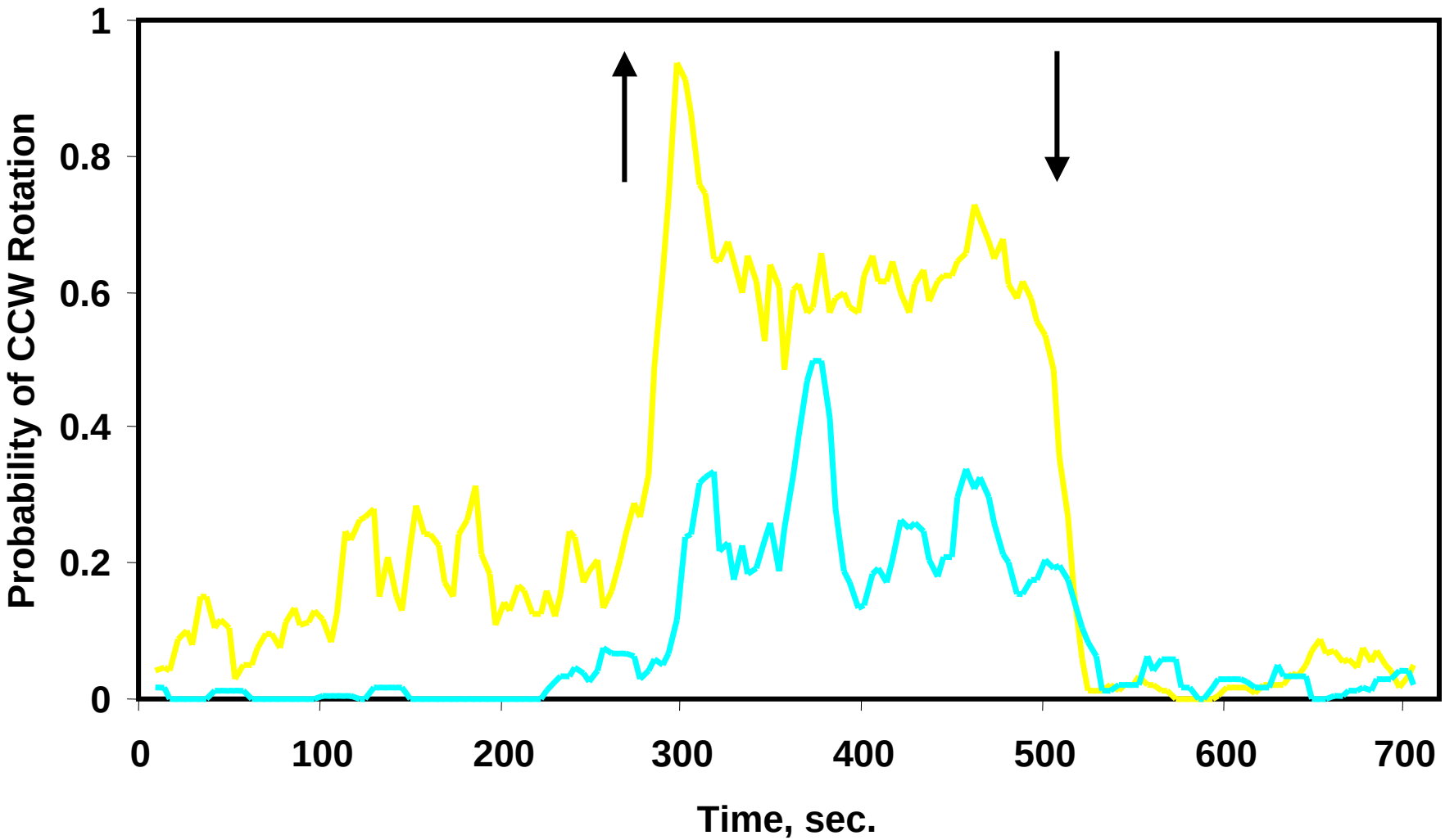
$$Bp \rightarrow B$$


CCW
Time
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60

Assumptions in Kinetic Models

- Homogeneous mixture (well mixed).
- Constant temperature and pressure.
- Single Phase.
- Intensive variables
  - average concentrations
  - statistical averages

Response of two different cells



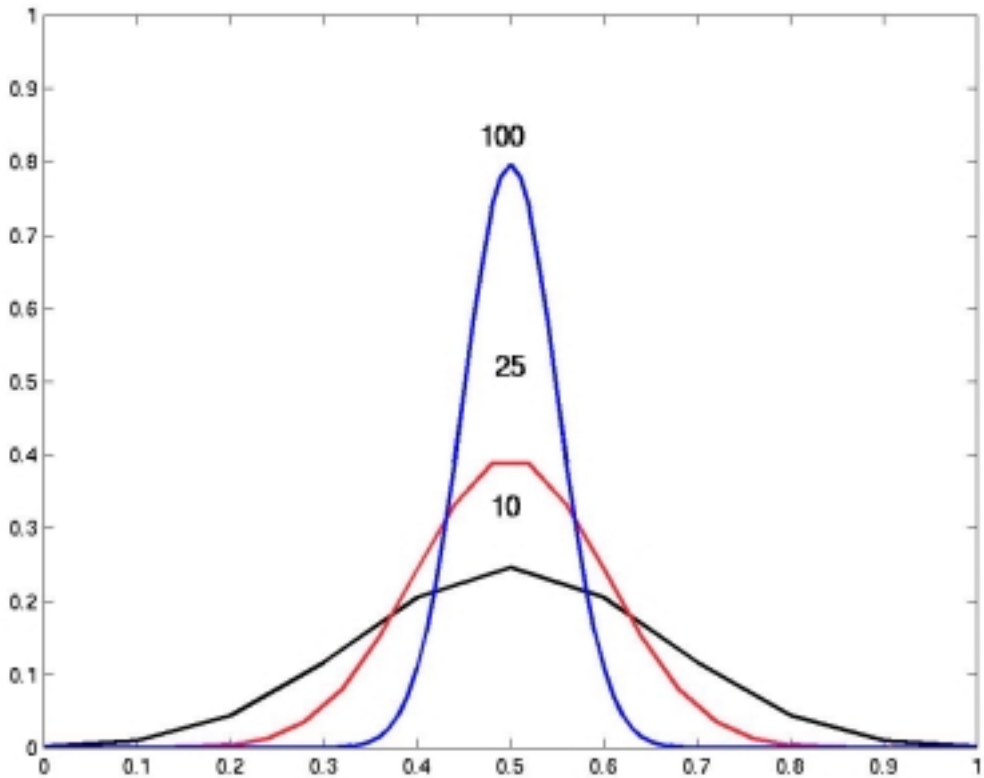
Deterministic vs. Probabilistic

Reaction Rate = Average number of reaction A in an infinitely small time interaval.

What about molecular fluctuations?

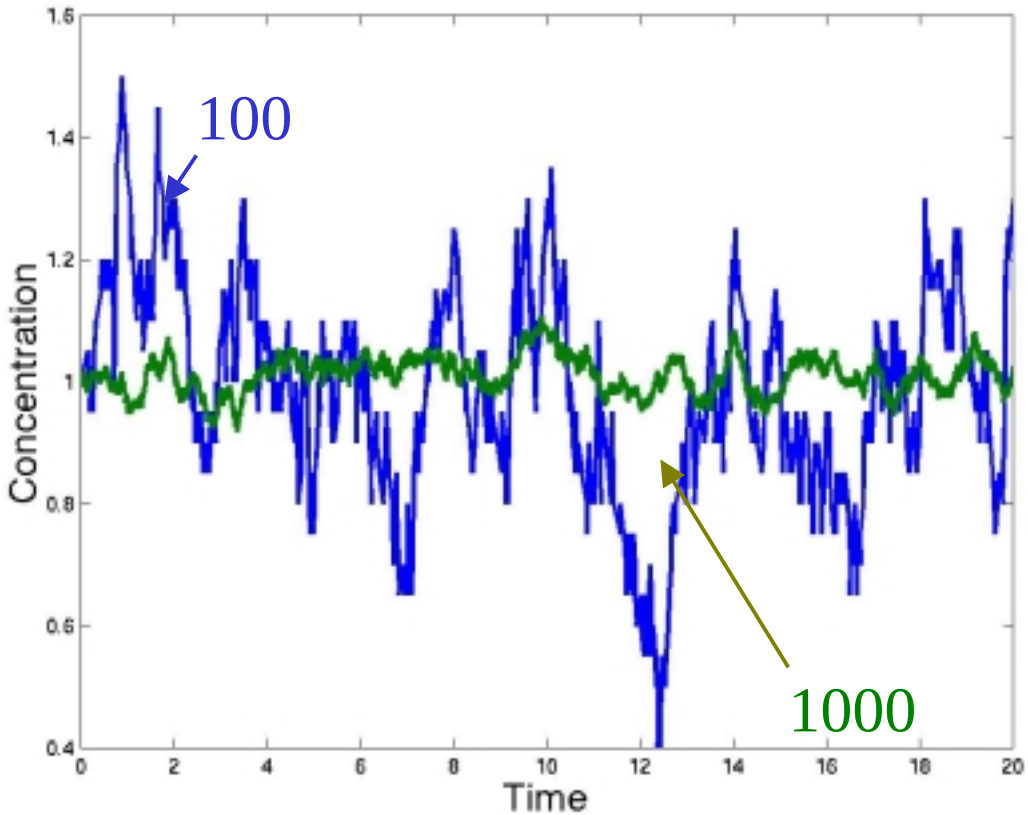
Reaction Rate = Relative likelihood of reaction A in an infinitely small time interaval.

Effect of Population Size
Coin Flipping Experiment



Dimerization Reaction

$$A \leftrightarrow B$$



Fluctuations scale roughly as square root of the population size

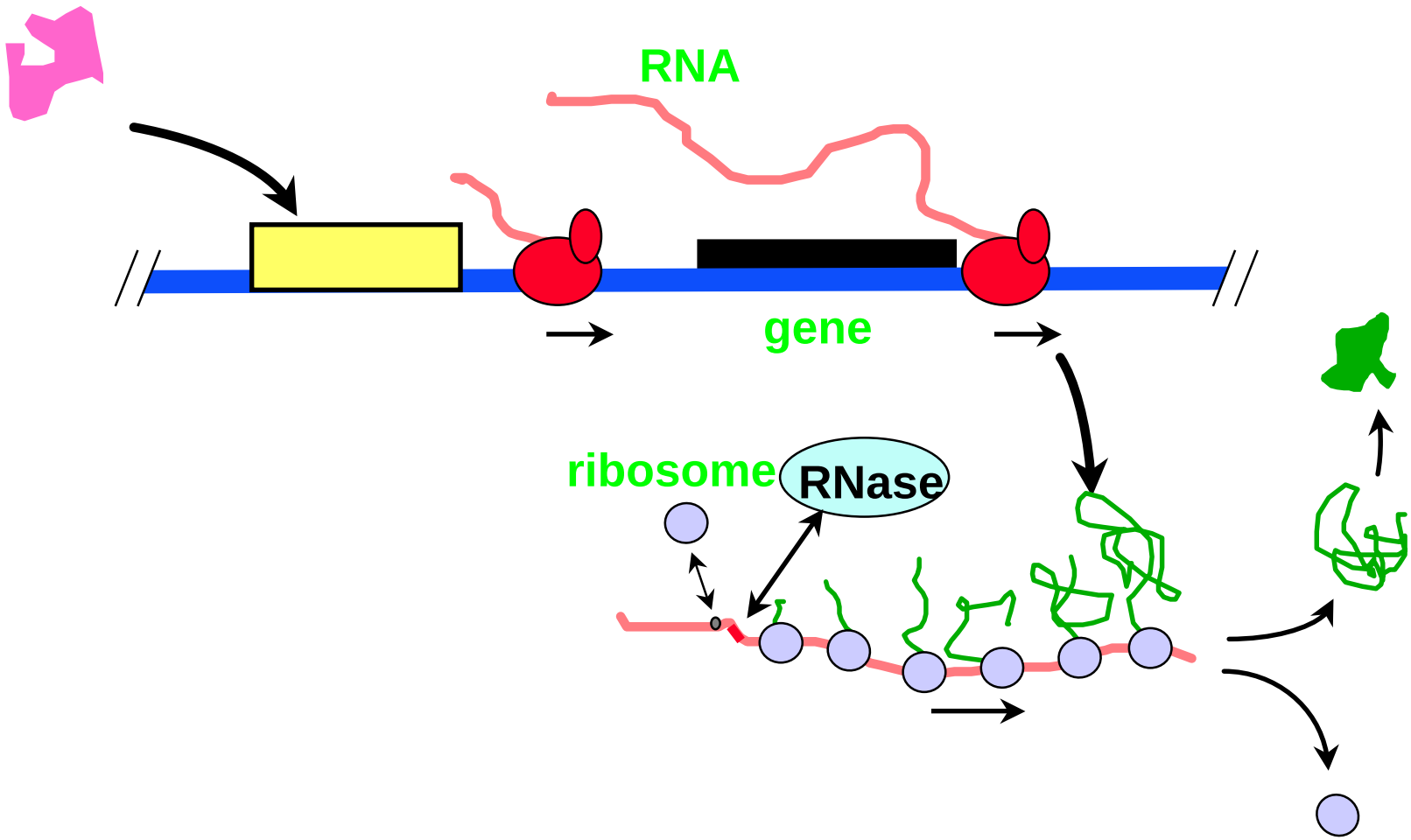
$$\text{std} \propto \frac{1}{\sqrt{N}}$$

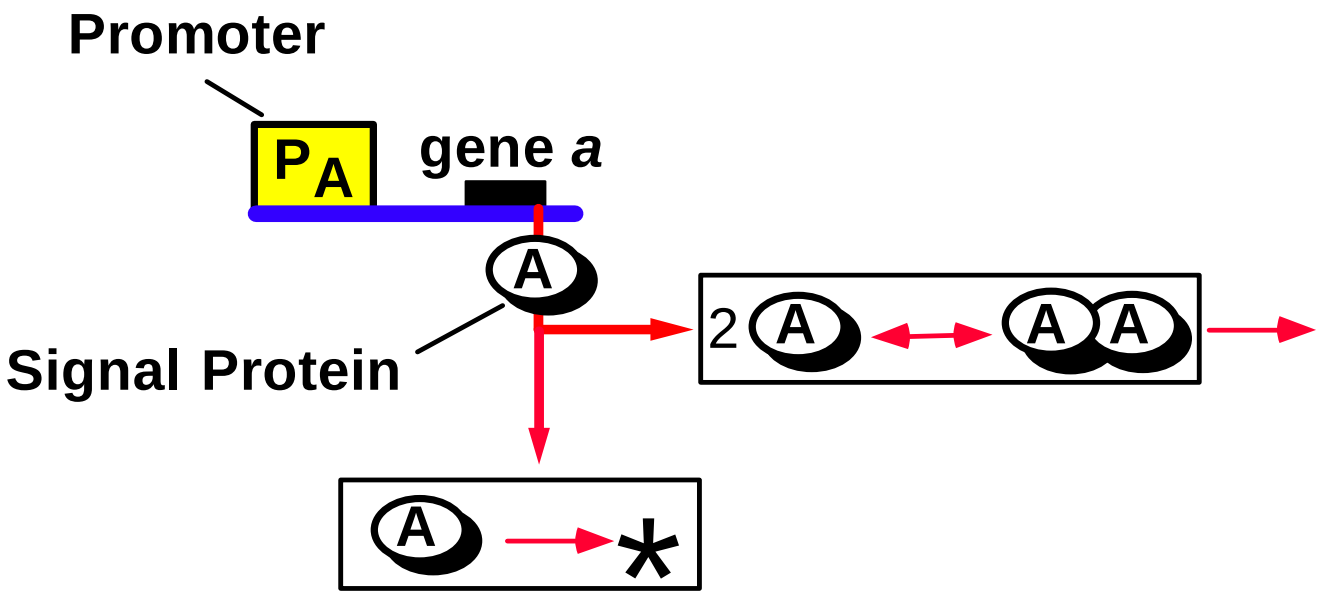
Cellular Environment

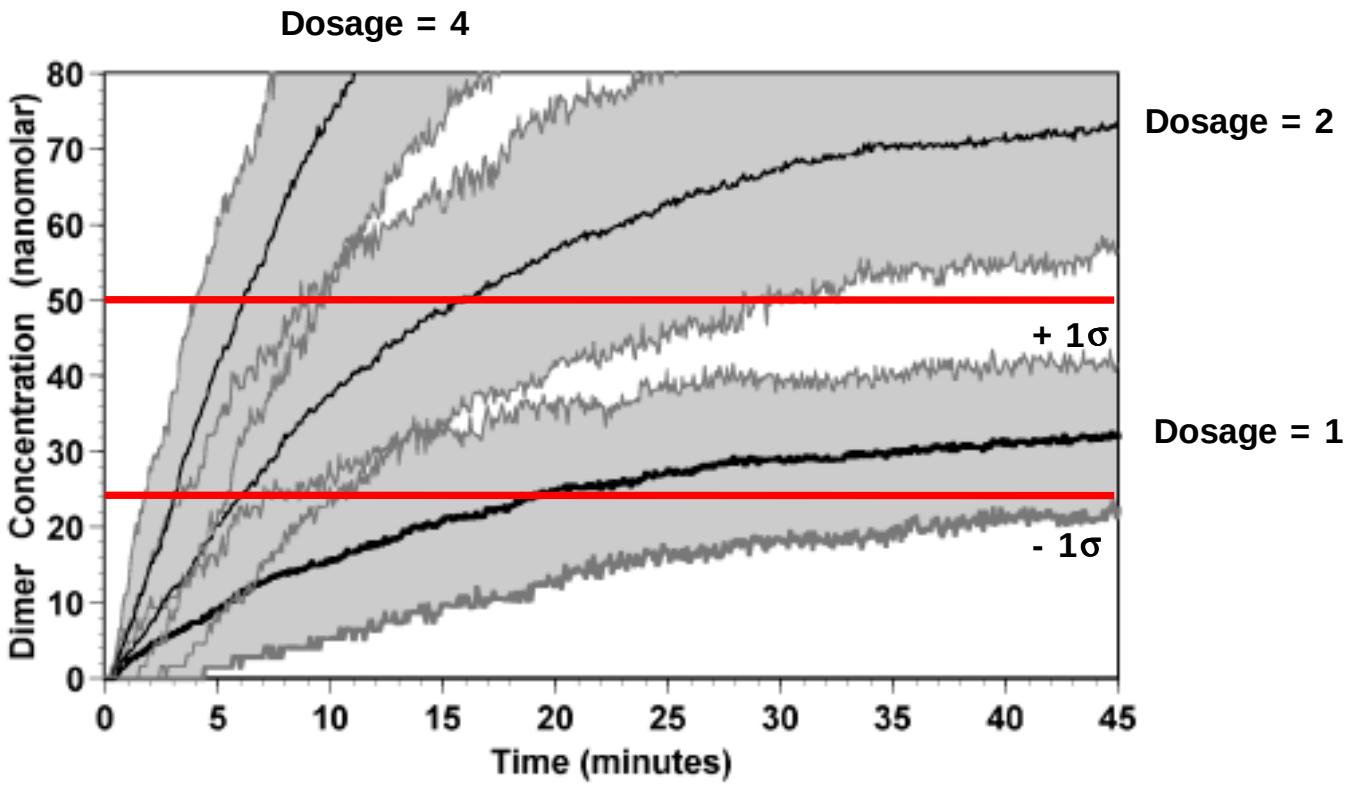
-Low concentrations (nanomolar)

-Single molecule events (DNA)

Gene Expression



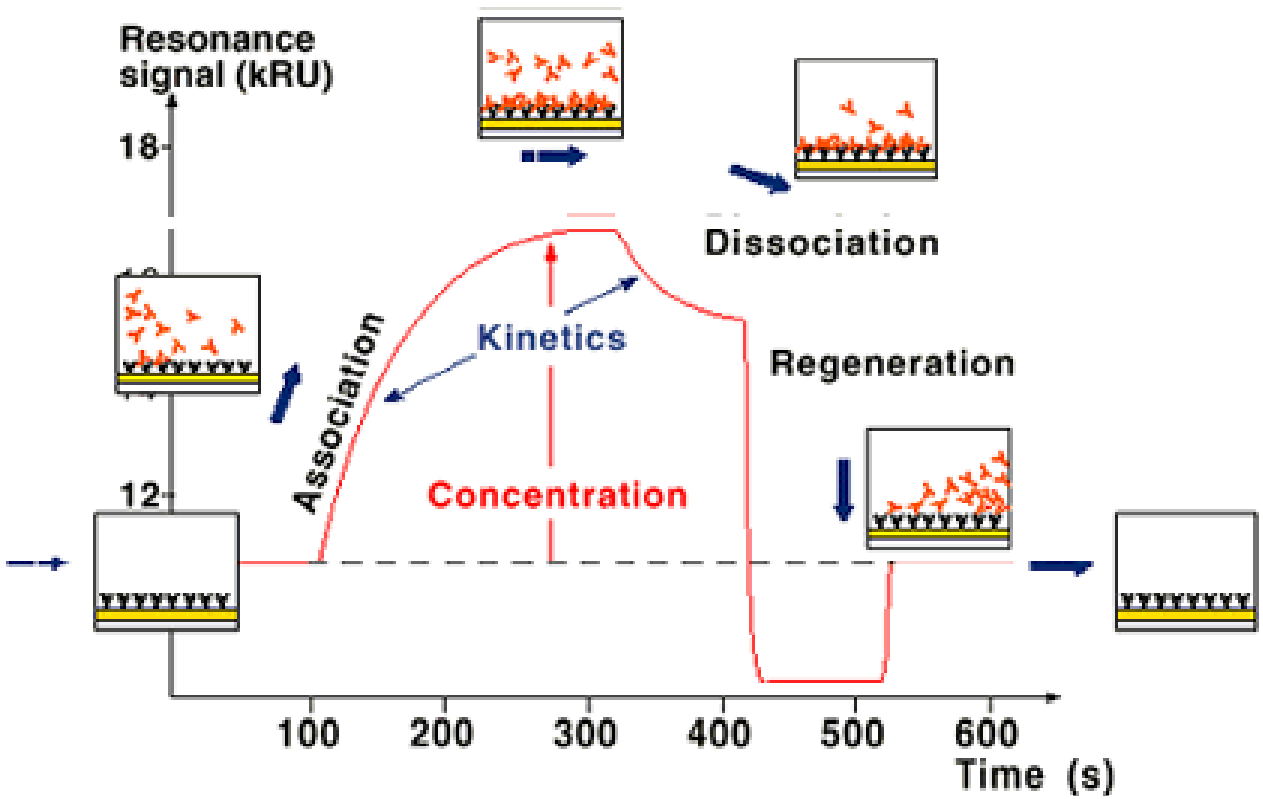

Promoter
P A
gene a
A
Signal Protein
2 A
A A
A
*

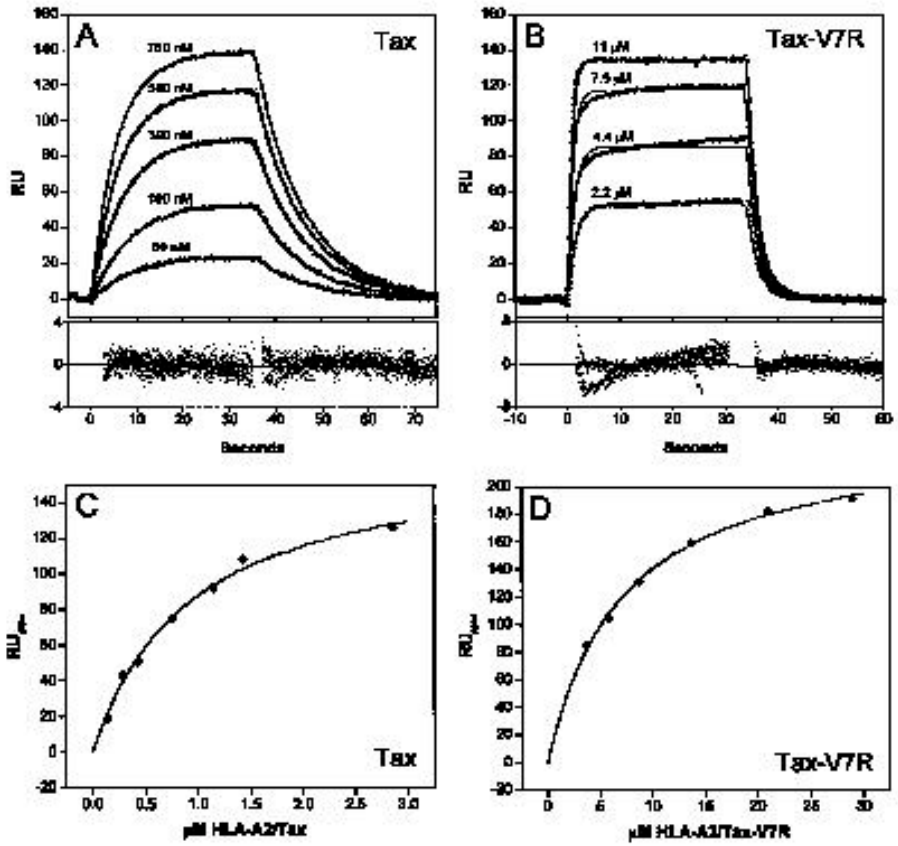

Dosage = 4
Dosage = 2
+ 1σ
Dosage = 1
- 1σ
Dimer Concentration (nanomolar)
Time (minutes)
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
35
40
45

How do we determine kinetics? Concentration measurements.

- Fluorescence
- Radioactivity
- Conductance
- Refraction
- *ab initio*
- etc….

Biacore


Resonance signal (kRU)
18
12
Association
Kinetics
Concentration
Dissociation
Regeneration
100
200
300
400
500
600
Time (s)



E

	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	$t_{1/2}$ (s)	k_{off}/k_{on} (μM)	K_{Dsurf} (μM)
Tax	$1.1 \pm 0.1 \times 10^5$	0.069 ± 0.002	7.5 ± 0.2	0.62 ± 0.06	0.91 ± 0.09
V7R	$6.6 \pm 0.9 \times 10^4$	0.47 ± 0.03	1.5 ± 0.1	8.1 ± 1.4	7.2 ± 0.5
P6A	n.d.	n.d.	–	–	n.d.
Y8A	n.d.	n.d.	–	–	n.d.